REMARKS

Claims 1-26 were pending before this response with claim 7 being withdrawn in accordance with a restriction requirement. By the present communication, the title of the invention, Abstract and paragraph numbers 0147, 0148, 0185, 0189 and 0194 in the Specification have been amended to overcome informalities as shown in attached Exhibit A. In addition, claims 7 and 20 have been canceled without prejudice and claims 1-3, 5, 6, 9, 21 and 23 have been amended as shown in attached Exhibit A. New claims 27-28 have been added. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly claims 1-6, 8-19 and 21-28 are currently pending.

Applicants wish to thank Examiner Loeb for the interview at the USPTO on June 4, 2002 with Applicants' representative Lisa Haile and for the Examiner's helpful suggestions.

The Restriction Requirement

Applicants confirm the provisional election made during a telephone conversation with Applicants' representative Lisa Haile on 25 January 2002 without traverse to elect prosecution of the invention of Group I (claims 1-6 and 8-26).

The Objection to the Specification

The Office Action enumerates various informalities in the disclosure that Applicants have addressed by amendment as follows:

In response to the objection to the title of the invention, the title has been amended to more closely reflect the subject matter of the elected claims. The amended title now reads:

The objection to the Abstract has been overcome by correction of the misspelling of "nucleic" in line 3.

Paragraph [0147] beginning on page 41 has been corrected by substitution of the symbol "β" for "(" at four locations.

Paragraph [0148] beginning on page 42 has been corrected by substitution of the symbol "β" for "(" at three locations.

Paragraph [0185] beginning on page 54 has been corrected by deletion of the sentence that refers to "Figure X" and correction of the typographical error in the phrase "which can the be decorated" to read instead: "which can then be decorated".

Paragraph [0189] beginning on page 56 has been corrected by substitution of the word "growth" for "grown".

Paragraph [0194] beginning on page 57 (mistakenly described by the Examiner as paragraph [0197]) has been corrected by deletion of "can" from the incorrect phrase "compounds can are utilized".

In response to the objection to the title of the invention, the title has been amended to more closely reflect the subject matter of the elected claims. The title now reads:

"METHOD FOR OBTAINING AN ENRICHED DNA SAMPLE CONTAINING ENZYMES OF SPECIFIED ACTIVITY"

Applicants respectfully submit that the above-described amendments to the Specification overcome the grounds for the objection to the Specification and reconsideration and withdrawal are respectfully requested.

The Objection to the Drawings

The Office Action indicates the drawings are objected to on various grounds. Applicants plan to submit new corrected Figures to overcome the objection to the drawings as follows: In Figure 7, the misspelling of "fluor" will be corrected; In Figure 14, "from host" will be replaced by "the library"; and in Figure 15, the misspelling of "growth" will be corrected. Applicants respectfully submit that the corrected Figures 7, 14 and 15 once submitted will overcome the grounds for the objection to the drawings.

Applicants respectfully request that the rejection regarding the figures be held in abeyance until allowance of the pending claims, at which time, formal drawings will be submitted.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claim 1-6 and 8-26 as allegedly being indefinite. With regard to the rejection of claim 1, Applicants disagree with the Examiner's assertion that failure to include a step that "clearly relates back to the preamble is a source of indefiniteness. However, to reduce the issues and expedite prosecution, claim 1 has been

amended to recite: "screening to recover the hybridized complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified activity in the recovered target DNA." Thus, Applicants submit that amended claim 1 clearly recites a step that relates back to the preamble.

With regard to the rejection of claim 2 for allegedly lacking antecedent basis for the term "recovered target DNA", Applicants submit that the term "the recovered target DNA" in amended claim 1 provides antecedent basis for the term at issue in claim 2.

With regard to the rejection of claim 3 for allegedly lacking antecedent basis for the term "the organisms", Applicants submit that amended claim 3 recites " the more than one organism is a plurality of microorganisms" in the place of "the organisms" so that antecedent basis is provided by the term "more than one organism" in claim 1.

With regard to the rejection of claim 5 for allegedly lacking antecedent basis for the phrase "the expression library," Applicants submit that claim 5 has been amended to depend from claim 2 in which the term "expression library" provides antecedent basis for the term at issue in claim 5. With regard to the rejection of claim 6 for allegedly lacking antecedent basis of the phrase "the DNA population," in amended claim 6 the phrase "target DNA obtained from the DNA population is selected by" has been deleted and replaced by the new phrase "the screening to recover the hybridized complementary sequences comprises", thus overcoming the grounds for the rejection of claim 6.

With regard to claim 9, the Examiner asserts that the term "derived from" is indefinite due to failure to recite "the number and nature of the derivative steps". To overcome the rejection the phrase "derived from" claim 9 has been deleted and replaced by the term "obtained from" which does not imply formation of derivatives.

In view of the above-described amendments and remarks, Applicants respectfully submit that claims 1-6 and 8-26 meet all requirements under 35 U.S.C. § 112, Second Paragraph. Reconsideration and withdrawal of the rejection is accordingly respectfully requested.

The Rejection under 35 U.S.C. § 102

Applicants respectfully traverse the rejection of claims 1-5 and 8-18, 20 and 24-26 as allegedly being anticipated by U.S. Patent No. 5,824,485 (hereinafter "Thompson") The invention methods for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample, as defined by amended claim 1, distinguish over the disclosure of Thompson by requiring a) co-encapsulating in a micro-environment selected from a liposome, gel microdrop, beads, agarose, cell, ghost red blood cell or ghost macrophage a one or a mixture of target DNA obtained from more than one organism with one or a mixture of DNA probes comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity; b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences; and c) screening to recover the hybridized complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified enzyme activity in the recovered target DNA.

Thompson fails to disclose each and every aspect of the invention methods as defined by amended claim 1. For example, Thompson fails to disclose co-encapsulation of the mixture containing the target DNA sequences and the probe in "a liposome, gel microdrop, beads, agarose, cell, ghost red blood cell or ghost macrophage, as recited in amended claim 1. In

addition, Thompson fails to disclose use of DNA probes that comprise a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity, as recited in amended claim 1. Thus, Thompson fails to disclose each and every aspect of the invention methods for enriching for target DNA sequences containing at least a partial coding region for an enzyme having a specified activity, such as a specified enzyme activity, as would be required to support a rejection for anticipation under 35 U.S.C. § 102. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection for alleged anticipation of claims 1-5 and 8-18, 20 and 24-26 over the disclosure of Thompson.

The Rejection Under 35 U.S.C. § 103

Applicants respectfully traverse the rejection of claims 1-6, 8-20 and 24-26 under 35 U.S.C. § 103 as allegedly being unpatentable over Thompson as applied to claims 1-5, 8-18, 20 and 24-26 in the rejection under 35 U.S.C. § 102 above and further in view of U. S. Patent 5,958,672 (hereinafter "Short"). Applicants respectfully submit that the invention methods for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample, as defined by amended claim 1, distinguish over the combined disclosures of Thompson and Short, by requiring a) co-encapsulating in a micro-environment selected from a liposome, gel microdrop, beads, agarose, cell, ghost red blood cell and ghost macrophage one or a mixture of target DNA obtained from more than one organism with one or a mixture of DNA probes comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity; b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences; and c) screening to recover the hybridized

complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified enzyme activity in the recovered target DNA.

The arguments above concerning the deficiencies of Thompson for disclosing the invention methods apply equally here. In addition, Thompson does not teach or suggest use of a micro-environment selected from a liposome, gel microdrop, beads, agarose, cell, ghost red blood cell or ghost macrophage to co-encapsulate a mixture of target DNA obtained from more than one organism with a mixture of DNA probes comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity.

Further, Applicants' method does not require that cells as described in Thompson be viable in order to perform Applicants' method. Since Applicants' method is based on determining hybridized DNA sequences, it is unnecessary to use viable cells for Applicants' screening method. In contrast, Thompson teaches encapsulation in "macro" droplets in order to provide enough space for proliferation of the cells within the droplet. (see col. 37, ll.38-67 bridging to col. 38, line 20). Thus, Thompson provides no motivation to use cells that need not be cultured under conditions that allow for viability.

The Examiner relies upon Short for disclosure regarding the various enzyme classes recited in pending claim 6. However, Short does not cure the above-described deficiencies in Thompson for suggesting the presently claimed invention because Short is completely silent regarding any type of co-encapsulating medium used to create a micro-environment for specific

interaction between "a mixture of target DNA obtained from more than one organism with a mixture of DNA probes comprising a detectable label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity", as recited in Applicants' claim 1, for example. Due to this silence in Short regarding use of co-encapsulation media for the purpose of separating out a group of DNAs that are enriched with respect to the proportion of clones that encode a specified activity, Applicants respectfully submit that Short fails to overcome the deficiencies of Thompson discussed above, so as to establish *prima facie* obviousness of Applicants' invention of claim 1 in combination with the teachings of Thompson.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-6, 8-19 and 24-26 under 35 U.S.C. § 103 as allegedly being unpatentable over the combined disclosures of Thompson and Short.

In view of the above amendments and remarks, reconsideration and favorable action on claims 1-6 and 17-19 and 21-26 are respectfully requested. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: 8/2/02

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Enclosure: Exhibit A

PATENT
ATTORNEY DOCKET NO.: DIVER1280-11

Applicants: Short and Keller
Application No.: 09/848,185
Filed: May 3, 2001
Art Unit: 1636
Examiner: Bronwen Loeb
Exhibit A: Page 1

EXHIBIT A

Version with Markings to Show Changes Made

In the Specification

Please delete the current title and substitute the following new title:

Please delete the current title and substitute the following amended title:

--METHOD FOR OBTAINING AN ENRICHED DNA SAMPLE CONTAINING ENZYMES OF SPECIFIED ACTIVITY--

Please amend the Abstract of the invention to read as follows:

(Amended) Disclosed is a process for identifying clones having a specified activity of interest, which process comprises (i) generating one or more expression libraries derived from [nuclei] nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a fluorescence activated cell sorter to identify said clones. More particularly, this is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; (ii) exposing said libraries to a particular substrate or substrates of interest; and (iii) screening said exposed libraries utilizing a fluorescence activated cell sorter to identify clones which react with the substrate or substrates. Also provided is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; and (ii) screening said exposed libraries utilizing an assay requiring co-encapsulation, a binding event or the

covalent modification of a target, and a fluorescence activated cell sorter to identify positive clones.

Please amend Paragraph [0147] beginning on page 41 to read as follows:

(Amended) Several methods have been described for using reporter genes to measure gene expression. These reporter genes encode enzymes not ordinarily found in the type of cell being studied, and their unique activity is monitored to determine the degree of transcription. Nolan *et al.*, developed a technique to analyze [(]β-galactosidase expression in mammalian cells employing fluorescein-di-(-D-galactopyranoside (FDG) as a substrate for [(]β-galactosidase, which releases fluorescein, a product that can be detected by a fluorescence-activated cell sorter (FACS) upon hydrolysis (Nolan *et al.*, 1991). A problem with the use of FDG is that if the assay is performed at room temperature, the fluorescence leaks out of the positively stained cells. A similar problem was encountered in other studies of [(]β-galactosidase measurements in mammalian cells and yeast with FDG as well as other substrates (Nolan *et al*, 1988; Wittrup *et al.*, 1988). Performing the reaction at 0°C appreciably decreased the extent of this leakage of fluorescence (Nolan *et al.*, 1988). However this low temperature is not adaptable for screening for, for instance, high temperature [(]β-galactosidases. Other fluorogenic substrates have been developed, such as 5-dodecanoylamino fluorescein di-(-D-galactopyranoside (C_{12}-FDG) (Molecular Probes) which differs from FDG in that it is a lipophilic fluorescein derivative that can easily cross most cell membranes under physiological culture conditions. The green fluorescent enzymatic hydrolysis product is retained for hours to days in the membrane of those cells that actively express the *lacZ* reporter gene. In animal cells C_{12}-FDG was a much better substrate, giving a signal which was 100 times higher than the one obtained with FDG (Plovins

et al., 1994). However in Gram negative bacteria like *E. coli,* the outer membrane functions as a barrier for the lipophilic molecule C_{12}-FDG and it only passes through this barrier if the cells are dead or damaged (Plovins et al). The fact that C_{12} retains FDG substrate inside the cells indicates that the addition of unpolarized tails may be used for retaining substrate inside the cells with respect to other enzyme substrates.

Please amend Paragraph [0148] on page 42 to read as follows:

(Amended) The abovementioned [(]β-galactosidase assays may be employed to screen single *E. coli* cells, expressing recombinant [(]β-D-galactosidase isolated from a hyperthermophilic archaeon such as *Sulfolobus solfataricus*, on a fluorescent microscope. Cells are cultivated overnight, centrifuged and washed in deionized water and stained with FDG. To increase enzyme activity, cells are heated to 70°C for 30 minutes and examined with a fluorescence phase contrast microscope. *E. coli* cell suspensions of the [(]β-galactosidase expressing clone stained with C_{12}-FDG show a very bright fluorescence inside single cells (Fig 8).

Please amend Paragraph [0185] on page 54 to read as follows:

(Amended) Probe nucleic acid sequences designed according to the method described above can also be utilized in the present invention to "enrich" a population for desirable clones. "Enrich", as utilized herein, means reducing the number and/or complexity of an original population of molecules. For example, probes are designed to identify specific polyketide sequences, and utilized to enrich for clones encoding polyketide pathways. [Figure X depicts

in-situ hybridization of encapsulated fosmid clones with specific probes of interest, in this case polyketide synthase gene probes.] Fosmid libraries are generated in E.coli according to the methods described in the Example herein. Clones are encapsulated and grown to yield encapsulated clonal populations. Cells are lysed and neutralized, and exposed to the probe of interest. Hybridization yields a positive fluorescent signal which can be sorted on a fluorescent cell sorter. Positives can be further screened via expression, or activity, screening. Thus, this aspect of the present invention facilitates the reduction of the complexity of the original population to enrich for desirable pathway clones. These clones can the be utilized for further downstream screening. For example, these clones can be expressed to yield backbone structures (defined herein), which can [the] then be decorated in metabolically rich hosts, and finally screened for an activity of interest. Alternatively, clones can be expressed to yield small molecules directly, which can be screened for an activity of interest. Further more, multiple probes can be designed and utilized to allow "multiplex" screening and/or enrichment. "Multiplex" screening and/or enrichment as used herein means that one is screening and/or enriching for more than desirable outcome, simultaneously.

Please amend Paragraph [0189] on page 56 to read as follows:

(Amended) *Streptomyces venezuelae*, unlike most other *Streptomyces* species, has been shown to sporulate in liquid [grown] growth culture. In some media, it also fragments into single cells when the cultures reach the end of vegetative growth. Because the production of most secondary metabolites, including bioactive small molecules, occurs at the end of log growth, it is possible to screen for *Streptomyces venezuelae* fragmented cells that are producing

bioactives by a fluorescence analyzer, such as a FACS machine, given the natural fluorescence of some small molecules.

Please amend Paragraph [0194] on page 57 to read as follows:

(Amended) In the method of the present invention, the fluorescing properties of this and other similar compounds [can] are utilized to screen for compounds of interest, as described previously, or are utilized to enrich for the presence of compounds of interest. Host cells expressing recombinant clones potentially encoding gene pathways are screened for fluorescing properties. Thus, cells producing fluorescent proteins or metabolites can be identified. Pathway clones expressed in E.coli or other host cells, can yield bioactive compounds or "backbone structures" to bioactive compounds (which can subsequently be "decorated" in other host cells, for example, in metabolically rich organisms). The "backbone structure" is the fundamental structure that defines a particular class of small molecules. For example, a polyketide backbone will differ from that of a lactone, a glycoside or a peptide antibiotic. Within each class, variants are produced by the addition or subtraction of side groups or by rearrangement of ring structures ("decoration" or "decorated"). Ring structures present in aromatic bioactive compounds are known in some instance to yield a fluorescent signal, which can be utilized to distinguish these cells from the population. Certain of these structures can also provide absorbance characteristics which differ from the background absorbance of a non-recombinant host cell, and thus can allow one to distinguish these cells from the population, as well. Recombinant cells potentially producing bioactive compounds or "backbone" structures can be identified and separated from a population of cells, thus enriching the population for desirable cells. Thus, the method of the present invention also facilitates the discovery of novel aromatic compounds encoded by gene

pathways, for example, encoded by polyketide genes, directly from environmental or other samples.

In the Claims

Please cancel claim 7 without prejudice.

Please amend claims 1-3, 5, 6, 9, 21 and 23 to read as follows:

1. (Amended) A method for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample comprising:

a) co-encapsulating in a micro-environment selected from a liposome, gel microdrop, bead, agarose, cell, ghost red blood cell and ghost macrophage a mixture of target DNA obtained from more than one organism with a mixture of DNA probes comprising a detectable [marker] label and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity;

b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences; and

c) screening to recover the hybridized complementary sequences containing the detectable label, thereby enriching the DNA sequences containing the at least partial coding region for the specified activity in the recovered target DNA.

2. (Amended) The method of claim 1, further comprising transforming host cells with the recovered target DNA to produce an expression library of a plurality of clones.

3. (Amended) The method of claim 1, wherein the [organisms are] more than one organism is a plurality of microorganisms.

5. (Amended) The method of claim [1] 2, further comprising screening the expression library for the specified enzyme activity.

6. (Amended) The method of claim 1, wherein the [target DNA obtained from the DNA population is selected by] screening to recover the hybridized complementary sequences comprises:

a) converting double stranded DNA into single stranded DNA;

b) recovering from the converted single stranded DNA, single stranded target DNA which hybridizes to probe DNA;

c) converting recovered single stranded target DNA to double stranded DNA; and

[c]d) transforming a host cell with the double stranded DNA of c).

9. (Amended) The method of claim 4, wherein the uncultured microorganisms are [derived] obtained from an environmental sample.

21. (Amended) The method of claim [20] 1, wherein the liposomes are prepared from one or more phospholipids, glycolipids, steroids, alkyl phosphates or fatty acid esters.

23. (Amended) The method of claim [20] 21, wherein the steroids are selected from the group consisting of cholesterol, cholestanol and lanosterol.